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Studies on the Control of Hyperacute Rejection in Hyperimmunized Rat : Combination of Donor Specific Blood Transfusion (DST) and Immunosuppressive Drugs

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ABSTRACT

In order to decrease preformed cytotoxic antibodies, which are the main cause of hyperacute rejection, donor specific blood transfusion (hereafter designated as DST) was performed. Immunosuppressants were administered at the same time to examine whether the combined treatment with DST can inhibit rapid reproduction of antibodies in the serum of a sensitized recipient.

Hyper immunized Fischer rat recipients were used as experimental models. Blood of ACI rat was transfused to the recipients as DST. Beta-methosone and Anti-lymphocyte serum (hereafter designated as ALS) were given as immuno suppressive drugs combined with DST.

The heart of ACI rat was transplanted to the hyperimmunized Fischer recipent treated as described above. The cardiac graft survival time was observed and the change in cytotoxic antibody titer of the recipient was determined with the elapse of time.

Performed cytotoxic antibodies formed by hyperimmunization were adsorbed or diminished, by DST, and the heart graft survivied for about 54 hr in the group treated with DST, while it was hyperacutely rejected after about 0.4 hr in controls.

However, DST was effective only when it was performed once. Transfusion after that acted as a booster, inducing reproduction of anti T-cell warm cytotoxic antibody (CA-TW). Therefore repeated transfusion was thought to be contraindication.

Beta-methasone or ALS were administered after adsorption of antibodies by DST in order to prevent antibodies from being rapidly formed again in the serum of a sensitized recipient. The suppressive effect was greatest in the group treated with combination of DST and ALS, and the heart graft survivied for 94 hr. In this group, the pattern of rejection was not hyperacute rejection but acute to accerelated one. It wa revealed that hyperacute rejection can be depressed to some extent.

If hyperacute rejection once occurs in organ transplantation, there has been no method to treat it, and the transplanted organ has been lost[4,10,15,32,40]. Various attempts have been done to decrease preformed cytotoxic antibodies which are the main cause of hyperacute rejection[6,25,36]. For example, blood dilution by physiological saline[25], adsorption by donors organ[28,36], and selective plasmapheresis by extracorporeal perfusion[25] have been tried to decrease the antibodies in the recipient serum. However, no successful results were obtained.

Although the antibody titer is dropped by these antibody adsorption procedure[18], mechanism of antibody production against the antigen remains in the individual once sensitized[40].

Therefore, when stimulation of antigen exists, the antibodies are produced again, after all inducing hyperacute rejection.

In this study, in order to search for possibility of controlling hyperacute rejection, we experimentally diminishes antibodies by DST, and administered immunosuppressants at the same time to deppress the mechanism of antibody production.

MATERIAL AND METHOD

A. Experimental Animal

Animals used were Fisher rats (RTI^l) and AC rat (RTI^a) rats weighing 150 to 300g, which are inbred strains and whose major histocompatibility antigens are incompatible[5,9]. ACI rats were used as donors and Fischer rats as recipients.

B. Experimental Procedure

1. Preparation of hyperimmunized Fischer recipient

The skin of ACI rat was transplanted to a Fischer rat. Spleen lymphord cell of ACI rat was intraperitoneally injected to a Fischer rat five time at intervals of two week after skin grafting to prepare hyperimmunized Fischer recipient[7].

2. Heterotopic heart transplantation

Heterotopic heart transplantation was performed according to method of Ono[30].

3. Assay of antibody titer

Lymphocyte was separated as previously described[7]. Antibody titer in the serum of Fischer rat was assayed according to method of Terasaki[38].

Assay of titer was performed just before donor specific blood transfusion, after two hr, and at intervals of 24 hr thereafter.

4. preparation of ALS

Blood of rat was collected by cardiopuncture under heparin treatment. Lymphocyte was separated by Ficoll-conray method. 5×10^8 lymphoid cells were mixed with 5cc of Freund's complete adjuvant and injected to the foot pat of rabbit. subsequently, 5×10^8 lymphoid cells were intravenously injected twice at intervals of two weeks. The blood was collected two weeks after the last immunization, the serum (ALS) separated from the collected blood was diluted ten fold and adsorbed by a sufficient volume of red blood cells of W.F. rat (volume 1.5 times the volume of ALS) before use[13].

C. Experimental Models

1. Controls

Hyperimmunized Fischer recipients were prepared by skin grafting and five booster shots of spleen lymphoid cell from ACI rat. The hearts of ACI rats were transplanted to the recipients, 10 ml/kg wt of physiological saline was intravenously administered as placebo.

2. Groups treated with donor specific blood transfusion

a) Groups treated with one shot of DST

As DST, peripheral blood of ACI rat was transfused to hyperimmunized Fischer recipients prepared by skin grafting and lymphoid cell booster from ACI rat. The animals were divided into the following groups according to the volume of transfused blood: non-DST group, group treated with 1.25 ml/kg wt of DST, group treated with 2.5 ml/kg wt of DST, group treated with 5.0 ml/kg wt of DST and group treated with 10 ml/kg wt of DST. The hearts of ACI rat were transplanted two hr after the blood transfusion.

b) Groups treated with one shot of Third Party Blood Transfusion (TPT)

Peripheral blood of W.F. rat was transfused to hyperimmunized Fischer recipients as TPT. The volume of transfused blood was as described above.

C) Groups treated with repeated DST

Peripheral blood of ACI rat was successively transfused to hyperimmunized Fischer rat recipients as DST once a day. The animals were divided into four groups according to the number of transfusion: non-DST group, group treated with DST once, group-treated with DST three times and group treated with DST six times. Each group was subdivided into three groups according to the volume of transfused blood: non-DST group, group treated with 1,25 ml/kg wt of DST and group treated with 2.5 ml/kg wt of DST. The hearts of ACI rat were transplanted to Fischer recipients two hr after the last DST.

3. Groups treated with combination of donor specific blood transfusion and immuno suppresive drug

a) Peripheral blood of ACI rat was transfused to hyperimmunized Fischer recipients prepared by skin grafting and lymphord cell booster from

ACI rat as DST.

The animals were divided into three groups according to the volume of DST: non-DST group, group treated with 1.25 ml/kg wt of DST and group treated with 2.5 ml/kg wt of DST. Each group was subdivided into four groups according to the dose of Beta-methasone: group not receiving, group receiving 0.25 mg/kg wt of Beta-methosone, group receiving 0.5 mg/kg wt of Beta-methosone and group receiving 1.0 mg/kg wt of Beta-methasone.

Beta-methasone was mixed with blood for DST and intravenously injected. The hearts of ACI rat were transplanted two hr after the intravenous injection. In order to counteract the differences in volumes of transfused blood and solvent of Beta-methasone, physiological saline was added to make up to a total volume of 10 ml/kg wt. The above described amounts of Beta-methasone were intravenously administered every day until the heart graft was rejected.

b) Groups treated with combination of DST and ALS

Peripheral blood of ACI rat was transfused to hyperimmunized Fischer recipients as DST.

The animals were divided into three groups according to the volume of DST: non-DST group, group treated with 1.25 ml/kg wt of DST and group treated with 2.5 ml/kg wt of DST. Each group was subdivided into four groups according to the dose of ALS: group not receiving, group receiving 0.125 ml/kg wt of ALS, group receiving 0.25 ml/kg wt of ALS, and group receiving 0.5 ml/kg of ALS.

In order to counteract the difference in the effect on the circulatory system resulting from the differences in volumes of transfused blood and administered ALS, physiological saline was added to make up to a total volume of 10 ml/kg wt. The above described amounts of ALS were intravenously administered every day until the heart graft was rejected.

RESULTS

1. Controls

The ACI heart transplanted to hyperimmunized Fischer rat was hyperacutely rejected in all recipients. The graft survival time was 0.46 ± 0.30 hr (M. ± S.D., n=10)

2. Groups treated with DST

a) Groups treated with one shot of DST

Blood of ACI rat was transfused to hyperimmunized Fischer recipients as preoperative DST, before the hearts of ACI rat were transplanted.

In the group treated with 1.25 ml/kg wt of DST, graft survival time was 31.8 ± 15.2 hr. T-test revealed that the graft survival time was significantly prolonged compared with 0.46 hr for controls ($p<0.05$)

In the group treated with 2.5 ml/kg wt of DST, graft survival time was 54 ± 20.0 hr (M. ± S.D., n=10), showing no significant difference from that of the group treated with 1.25 ml/kg wt. One of the ten recipients died before the heart graft was rejected.

In the group treated with 5.0 ml/kg wt of DST, six of the ten recipients died of multiple thrombosis or bleeding. When the survival period of animal was assumed to be graft survival time in the dead recipients, graft survival time of this group was 35 ± 37.8 hr.

In the group treated with 10 ml/kg wt of DST, nine of the ten recipients died of multiple thrombosis or bleeding. The mean graft survival time was 7.6 ± 7.0 hr (Fig. 1).

b) In the groups treated with peripheral blood of W.F. rat (TPT) as controls for DST.

Although there was a significant difference in graft survival time as shown in Table 1, the effect was hardly observed.

c) Groups treated by repeated DST

Graft survival time was 24.3 + 8.8 hr in the

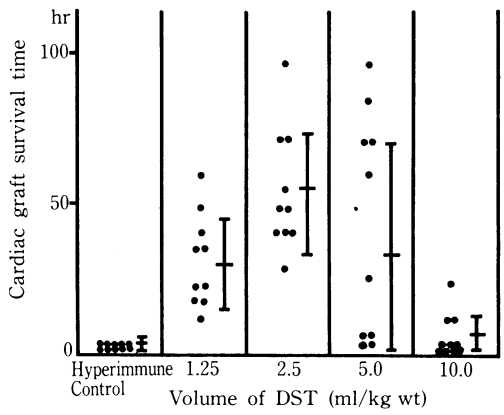

Fig. 1. Duration of cardiac graft survival and Donor Specific Blood Transfusion.
A significant difference was observed between the control group and the 1.25 to 2.5ml/kg wt DST group. ($p<0.05$)

Table 1. Duration of cardiac graft survival and Third Party Blood Transfusion (TPT)

Volume of TPT	Graft survival time
Control	0.46±0.3hr
1.25ml/kg wt	1.5 ±2.5hr
2.5 ml/kg wt	2.5 ±1.3hr§
5.0 ml/kg wt	2.4 ±0.9hr§

§A significant difference was observed between the control group and the TPT group. ($p<0.01$)

group treated with 1.25 ml/kg wt of DST three times (n=6) and 45.3 ± 10.7 hr in the group treated with 2.5 ml/kg wt of DST three times. The graft survival times of both groups were abbreviated compared with those for the respective groups treated once. However, no significant difference ($p>0.05$) was revealed in t-test (Table 2).

Table 2. Duration of cardiac graft survival and Frequency of daily transfusion of DST

Volume of transfusion	Frequency of DST	Graft survival time
Control	0	0.46±0.3hr§
1.25ml/kg wt	1	31.8±15.2hr
	3	24.3± 8.8hr
	6	2.8± 1.4hr§
2.5ml/kg wt	1	54.0±20.0hr
	3	45.3±10.7hr
	6	0.8± 0.6hr§

§ cardiac grafts were rejected hyperacutely.

Table 3. Changes in cytotoxic antibody after DST

DST	CA-TW
Before	8.5±0.9
2 hr after	4.5±0.7§
1 day after	4.7±0.6§
2 days after	4.6±4.8§
3 days after	9.9±1.1
4 days after	9.7±0.9
5 days after	10.1±1.1
6 days after	10.1±1.1
7 days after	10.5±1.0

§Significant difference noted as compared to before DST. ($p<0.01$)
Titer expresses dilution in multiples of two.
DST: Donor Specific Blood Transfusion

Table 4. Changes in cytotoxic antibody following daily transfusion of DST

Daily DST	CA-TW
Before	8.2±1.2
2 hr	4.6±0.6§
1 day	4.7±0.9§
2 days	8.2±1.0
3 days	8.9±1.3
4 days	11.0±1.3
5 days	12.9±1.1
6 days	15.4±1.0
7 days	16.2±1.0

§Significant difference noted as compared to before DST. ($p<0.01$)
Titer expresses dilution in multiples of two.

On the other hand, graft survival time of the group treated with 1.25 ml/kg wt of DST six times was 2.8 ± 1.4 hr (n=6) and that of the group treated with 2.5 ml/kg wt of DST six times was 0.8 ± 0.6 hr (n=6). The heart graft was hyperacutely rejected in all recipients of both the groups (Table 2).

d) Change in cytotoxic antibody

1) Effect of one shot of DST on preformed cytotoxic antibody

Table 3 show the change in cytotoxic antibody titer in the group treated by 2.5 ml/kg wt of DST once which showed the most favorable effect of prolonging cardiac graft survival time. CA-TW titer was significantly dropped for two days after transfusion ($p<0.01$). However, the antibody was rapidly formed again after that.

2) Effect of repeated DST on preformed cytotoxic antibody

Table 4 shows the change in cytotoxic antibody titer in the group treated by 2.5 ml/kg wt of DST successively.

CA-TW titer was significantly dropped up to 24 hr after transfusion ($p<0.01$). It rose rapidly after the second DST, showing that the second or the later DST acted as booster.

3. Groups treated with combination of DST and immuno-suppressive drugs

Hyperimmunized Fischer rat recipients prepared by skin grafting and five booster shots of spleen lymphoid cell from ACI rat underwent transfusion of blood of ACI rat as preoperative

DST and administration of Beta-methasone or ALS, before the hearts of ACI rat were transplanted.

a) Groups treated with combination of DST and Beta-methasone

1) Graft survival time

Of the groups treated with Beta-methasone alone, graft survival time was 1.47 ± 0.83 hr in the group treated with 0.25 mg/kg wt of Beta-methasone (n=7), 1.51 ± 0.51 hr in the group treated with 0.5 mg/kg wt (n=7) and 1.96 ± 0.82 hr in the group treated with 1.0 mg/kg wt (n=7). Graft survival time tended to be prolonged with the increase in dose of Beta-methasone, being significantly prolonged compared with 0.46 ± 0.30 hr for controls ($p<0.05$).

Table 5. Effects of combined use of DST and Betamethasone for hyperacute rejection

Volume of DST	Administration of Beta-methasone	Graft survival time
0.00ml/kg wt	0.00mg/kg wt	0.46±0.30hr
	0.25mg/kg wt	1.47±0.83hr
	0.50mg/kg wt	1.51±0.51hr
	1.00mg/kg wt	1.96±0.82hr
1.25ml/kg wt	0.00mg/kg wt	31.8±15.4hr
	0.25mg/kg wt	40.2± 9.6hr
	0.50mg/kg wt	44.5±11.9hr
	1.00mg/kg wt	49.7±15.7hr
2.50ml/kg wt	0.00mg/kg wt	54.0±20.0hr
	0.25mg/kg wt	58.2±15.7hr
	0.50mg/kg wt	52.2±10.2hr
	1.00mg/kg wt	63.6±46.3hr

A significant difference was not observed between the DST and Beta-methasone combined group and the DST only group.

However, the heart graft wa hyperacutely rejected in all recipients (Table 5).

There was no significant difference in graft survival time between the groups treated with combination of DST and Beta-methasone and the group treated with DST alone (Table 5).

2) Change in cytotoxic antibody

Table 6 shows the time-course of cytotoxic antibody titer of the group treated with combination of 2.5 ml/kg wt of donor blood and 0.5 mg/kg wt of Beta-methasone.

The time of CA-TW was significantly decreased by administration of DST and Beta-methasone for two days ($p<0.01$). Thereafter the antibody was rapidly produced again.

b) Groups treated with combination of DST and ALS

Table 6. Changes in cytotoxic antibody following combined use of DST and Beta-methasone

DST and Beta-methasone	CA-TW
Before	8.3±1.2
2 hr after	4.4±1.0§
1 day	4.2±0.9§
2 days	4.0±0.8§
3 days	7.7±1.1
4 days	8.4±1.3
5 days	8.4±1.3
6 days	8.6±1.5
7 days	9.4±1.6

§Significant difference noted as compared to before combined use of DST and Beta-methasone. ($p<0.01$)

Titer expresses dilution in multiples of two.

1) Graft survival time

In the groups of hyperimmunized Fischer recipients receiving ALS without preoperative DST, graft survival times were 0.46 ± 0.30 hr (n=10), 0.61 ± 0.45 hr (n=7), 0.92 ± 0.55 hr (n=7) and 0.90 ± 0.62 (n=7) for the groups receiving 0, 0.125, 0.25 and 0.5 ml/kg wt of ALS, respectively. There was no significant difference among the groups ($p>0.05$). That is, when ALS was administered alone, no suppressive effect on hyperacute rejection was observed (Table 7).

Of the groups treated with combination of DST and ALS. the groups treated with 1.25 ml of DST and ALS showed following results as to the prolonging effect on graft survival time: Graft survival times were 31.8 ± 15.4 hr (n=10), 42.8 ± 15.2 hr (n=7) and 54 ± 15.4 hr (n=7) for the groups receiving 0, 0.125 and 0.25 ml/kg wt of ALS respectively, and no significant difference was observed ($p>0.05$). However, graft survival time of the group receiving 0.5 ml/kg wt of ALS was 59.2 ± 9.3 hr (n=8), being significantly prolonged compared with for the group treated with DST alone ($p<0.05$).

Table 7. Effects of combined use of DST and ALS for hyperacute rejection

Volume of DST	Administration of ALS	Graft survival time
0.00ml/kg wt	0.00 ml/kg wt	0.46±0.30hr
	0.125ml/kg wt	0.61±0.45hr
	0.25 ml/kg wt	0.92±0.55hr
	0.5 ml/kg wt	0.90±0.62hr
1.25ml/kg wt	0.00 ml/kg wt	31.8±15.4hr
	0.125ml/kg wt	42.8±15.2hr
	0.25 ml/kg wt	54.0±11.4hr
	0.5 ml/kg wt	59.2± 9.3hr§
2.5ml/kg wt	0.00 ml/kg wt	54.0±20.0hr
	0.125ml/kg wt	70.2±20.1hr
	0.25 ml/kg wt	80.0±18.6hr
	0.5 ml/kg wt	94.8±17.3hr§

§A significant difference was observed between the DST and ALS combined group and the DST only gruop. ($p<0.05$)

The prolonging effect of combination of 2.5 ml of DST and ALS on survival time was as follows: Graft survival times were 54.0 ± 20.0 hr (n=10), 70.2 ± 20.1 hr (n=7) and 80.0 ± 18.6 hr (n=7) for the groups receiving 0, 0.125 and 0.25 ml/kg wt of ALS, respectively, and no significant difference was observed ($p>0.05$). Graft survival time of the group receiving 0.5 ml/kg wt of ALS was 94.8 ± 17.3 hr (n=7), being significantly prolonged compared with for the group treated with DST alone ($p<0.05$) (Table 7).

2) Change in cytotoxic antibody

Table 8 shows the time-course of cytotoxic antibody titer of the group treated with combination of 2.5 ml/kg wt of donor blood and 0.5 ml/kg wt of ALS, which showed the most favorable graft survival time

The titer of CA-TW was significantly dropped by combined administration of DST and ALS for four days ($p<0.01$).

Table 8. Changes in cytotoxic antibody following combined use of DST and ALS

DST and ALS	CA-TW
Before	7.9±0.9
2 hr after	4.7±0.8§
1 day	4.4±1.1§
2 days	4.2±1.2§
3 days	4.5±1.5§
4 days	4.6±1.2§
5 days	9.1±1.4
6 days	10.5±1.5
7 days	10.6±1.2

§Significant difference noted as compared to before combined use of DST and ALS. ($p<0.01$)
Titer expresses dilution in multiples of two.

DISCUSSION

In order to inhibit hyperacute rejection, following methods have been examined in animal experiments: administration of heparin for preventing intravascular coagulation[10,29,34], adsorption of cytotoxic antibodies by perfusion using donor organ[16,36], blood dilution[26] or selective plasmapheresis to reduce the IgG fraction including the antibodies[25], methods of lowering complement titer by cobra venom factor[16,37], citrate[19,21] or EDTA[31], perfusion of renal graft by F(ab')[12,16], and administration of anti-lymphocyte globulin[13].

In clinical investigations, administration of heparin[10,15,41], adsorption of cytotoxic antibodies using donor organ[41], perfusion of renal graft by F(ab')[4] and administration of citrate[4] have been tried.

A search of the literature revealed that the most favorable suppressive effect on hyperacute rejection was observed in an experiment transplanting the pig kidneys to dogs. In the experiment, citrate was continuously infused into the artery. It was reported that the mean renal graft survival time was 22 hr[29]. However, there has been no report of animal experiment or no case report at all which could prolong the mean graft survival time longer than 24 hr.

Many reports described that donor specific blood transfusion (abbreviated as DST) was effective in prolonging survival time of transplanted kidney[20,24,35,39]. These attempts were performed in order to improve the results of take of graft in ordinary patients not sensitized. There has been no report that DST was used to control hyperacute rejection.

In order to investigate the effect of DST on hyperacute rejection, we carried out the present study. First, we performed Third Party Blood Transfusion (TPT) as a control experiment for DST; blood of W.F. rat was transfused to Fischer recipients hyperimmunized by ACI antigens.

Which heart graft was hyperacutely rejected 0.46 ± 0.30 hr after grafting in the group of non-treated controls, cardiac graft survival time of the group treated with TPT was 2.5 ± 1.3 hr, being significantly prolonged. Although it was reported that TPT was effective against acute rejection in dog[2], there had been no information as to TPT performed against hyperacute rejection.

In our experiment on suppressive effect of DST on hyperacute rejection, cardiac graft survival time of the group treated with 2.5 ml/kg wt of DST was prolonged to 54.0 ± 20.0, indicating marked suppressive effect on hyperacute rejection.

Further, the rejections observed in the groups treated with DST were not hyperacute ones for which there is no suppressing method, but accelerated or acute ones for which there is a possibility of being depressed to some extend by administration of immuno-suppressive drug before and after transfusion. This observation is also an important effect of DST.

Although there has been no attempt conducting DST against hyperacute rejection in literature, Mozes reported an experiment in which tissue of donor was intravenously injected before transplantation to adsorb antibody. The kidney was transplanted from pig to dog in his experiment. The renal graft was hyperacutely rejected three to five min after grafting in non-treated recipients. When lyophilized lymphatic tissue of pig was intravenously injected before the transplantation, graft survival time was prolonged to 60 min[28].

The dose is another important problem in using DST to control hyperacute rejection. When a large dose of DST (5.0 or 10.0 ml/kg wt) was administered, recipients died probably because of excessive antigen-antibody reaction. Bleeding and multiple thrombus in the blood vessels of various organs were observed in the dead recipients.

The optimum dose of DST for rat was thought to be 2.5 ml/kg wt from our results. In order to obtain the maximum effect of DST, it will be important to decide the dose of blood transfusion which gives suppressive effect on hyperacute rejection and not induces bleeding or multiple thrombus.

It is reported by Janasen that cytotoxic antibody of mouse sensitized by hemologous mouse antigen was adsorbed and decreased by erythrocyte of donor mouse *in vitro*[14].

When the effect of DST on cytotoxic antibody was analyzed in our experiment, CA-TW titer was significantly dropped after blood transfusion in the groups treated with DST ($p<0.01$). The effect of DST which prolonged cardiac graft survival time can be ascribed mainly to adsorption of CA-TW which is the major cause of hyperacute rejection.

In the group treated with 2.5 ml/kg wt of DST which showed the most favorable prolonging effect on cardiac graft survival time, cytotoxic antibody titer was dropped only for two days, and the antibody was quickly produced again after that. The duration of effect of one shot of DST was thought to be very short.

Expecting that longer graft survival will be obtained when DST is repeated and antibody is absorbed successively, we conducted repeated DST. However, graft survival time was shortened with the increase in the number of DST. The change of cytotoxic antibody in the groups treated with repeated DST showed that the antibody titer was rapidly raised by the second or later blood transfusion repeated every day. It became clear that the blood transfused at the second time or later do not absorb the antibody but acts as booster.

It was thought from these findings that if any treatment was not performed on the antibody producing system, the antibody titer rose again and hypercute rejection occurred in spite of adsorption of antibody. Therefore we anticipated that hyperacute rejection could be depressed for longer time if antibody was absorbed and decreased by DST and reproduction of the antibody was inhibited by immunosuppressant at the same time. Beta-methasome and Anti lympocyte serum (hereafter abbreviated as ALS) were employed as immunosuppressive drugs used with DST.

There has been no report on attempts to depress hyperacute rejection by combinated administration of DST and steroids in animals. As to clinical investigation, Williams et al reported that hyperacute rejection could not be prevented by blood transfusion during surgery and administration of a large amount of steroids[41]. However no report has described preventive ef-

fect of combined use of preoperative DST and a large dose of steroids on hyperacute rejection.

In our study, the suppressive effect on hyperacute rejection was very slight in the group treated Beta-methasone alone, as in the animal experiment[37] and the case report reported before[41]. Combinated use of DST and Beta-methasone did not show a significant prolongation of graft survival time compared with the group treated with DST alone.

Since there have been no other animal experiment or case report, we can not make a comparative analysis of the effect of combined use of DST and Beta-methasone on cytotoxic antibody. However, our study showed that CA-TW titer was significantly dropped for two days. This duration of decreased titer was almost equal to that for the group treated with DST alone. This fact was thought to be the reason that combination of DST and Beta-methasone did not give the better effect.

Next, we tried to depress hyperacute rejection by using a more powerful suppressive drug, ALS, with DST.

Administration of ALS alone was not effective against hyperacute rejection in our experiment. Hammer reported that renal graft survived for 8 hr when horse anti dog ALG was used in an experiment prefusing cat kidney by canine blood[13]. On the other hand, Buttman described that treatment by ALS alone was not effective at all against hyperacute rejection in rat[11], as in our experiment.

As to the effect of combinated use of DST and ALS on hyperacute rejection, the present study showed that graft survival time was significantly prolonged upto 94.8 ± 17.3 hr with the increases of doses of transfused blood and ALS compared with 0.46 ± 0.30 hr for controls, revealing marked suppressive effect on hyperacute rejection.

Since there have been no other report on animal experiment nor clinical investigation as to the change in cytotoxic antibody under combined administration of DST and ALS, we cannot make a comparative analysis. However, CA-TW titer was dropped for four days by combined use of DST and ALS in our study. This finding showed that the combination could delay the re-rise of antibody titer two days longer than administration of DST alone.

Survival time of heart graft of the group treated with combination of DST and ALS was 94.8 ± 17.3 hr in hr in our method. Compared with other method[1,11,12,16,19,26] by which hyperacute rejection was depressed for 24 hr or shorter, our method was thought to be markedly effective against hyperacute rejection.

We presented an approach to hyperacute rejection which has been thought to be unable to be controlled at all. We anticipate that our findings will be a helpful guide to treatment of hyperacute rejection in clinical cases.

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